

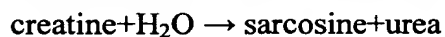
## AMENDMENTS

### *IN THE SPECIFICATION:*

Please replace the paragraph at column 2, lines 33-47 with:

Accordingly, the present invention provides a novel creatine amidinohydrolase having the following physicochemical properties.

Action: catalyzing the following reaction:



Optimum temperature: ca. 40-50° C

Optimum pH: ca. 8.0-9.0

Heat stability: stable at not more than about 50° C (pH 7.5, 30 min)

Km value relative to creatine in a coupling assay using a sarcosine oxidase and a peroxidase: ca. 3.5-10.0 mM

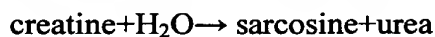
Molecular weight: ca. 43,000 (SDS-PAGE)

Isoelectric point: ca. ~~3.5~~ 4.5

Please replace the paragraph at column 2, lines 48-64, with:

The present invention also provides a method for producing said creatine amidinohydrolase, comprising culturing a microorganism capable of producing a novel creatine amidinohydrolase having the following physicochemical properties, in a nutrient medium, and harvesting said creatine amidinohydrolase from the culture.

Action: catalyzing the following reaction:



Optimum temperature: ca. 40-50° C

Optimum pH: ca. 8.0-9.0

Heat stability: stable at not more than about 50°C (pH 7.5, 30 min)

Km value relative to creatine in a coupling assay using a sarcosine oxidase and a peroxidase: ca. 3.5-10.0 mM

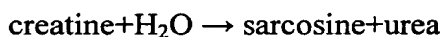
Molecular weight: ca. 43,000 (SDS-PAGE)

Isoelectric point: ca. ~~3.5~~ 4.5

Please replace the paragraph at column 3, lines 21-35, with:

One embodiment of the present invention is a novel creatine amidinohydrolase having the following physicochemical properties.

Action: catalyzing the following reaction:



Optimum temperature: ca. 40-50° C

Optimum pH: ca. 8.0-9.0

Heat stability: stable at not more than about 50° C (pH 7.5, 30 min)

Km value relative to creatine in a coupling assay using a sarcosine oxidase and a peroxidase: ca. 4.5±1.0 mM

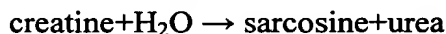
Molecular weight: ca. 43,000 (SDS-PAGE)

Isoelectric point: ca. ~~3.5~~ 4.5

Please replace the paragraph at column 3, lines 36-49, with:

Another embodiment of the present invention is a novel creatine amidinohydrolase having the following physicochemical properties.

Action: catalyzing the following reaction:



Optimum temperature: ca. 40-50° C

Optimum pH: ca. 8.0-9.0

Heat stability: stable at not more than about 50° C (pH 7.5, 30 min)

Km value relative to creatine in a coupling assay using a sarcosine oxidase and a peroxidase: ca. 6.5±1.0 mM

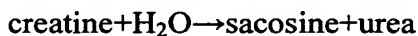
Molecular weight: ca. 43,000 (SDS-PAGE)

Isoelectric point: ca. ~~3.5~~ 4.5

Please replace the paragraph at column 3, lines 50-63, with:

A still another embodiment of the present invention is a novel creatine amidinohydrolase having the following physicochemical properties.

Action: catalyzing the following reaction:



Optimum temperature: ca. 40-50° C

Optimum pH: ca. 8.0-9.0

Heat stability: stable at not more than about 50° C (pH 7.5, 30 min)

Km value relative to creatine in a coupling assay using a sarcosine oxidase and a peroxidase: ca. 9.0±1.0 mM

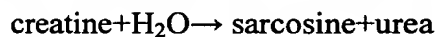
Molecular weight: ca. 43,000 (SDS-PAGE)

Isoelectric point: ca. ~~3.5~~ 4.5

Please replace the paragraph at column 5, lines 3-16, with:

The novel creatine amidinohydrolase obtained by the above-mentioned production method of the present invention has the following physicochemical properties.

Action: catalyzing the following reaction:



Optimum temperature: ca. 40-50° C

Optimum pH: ca. 8.0-9.0

Heat stability: stable at not more than about 50° C (pH 7.5, 30 min)

Km value relative to creatine in a coupling assay using a sarcosine oxidase and a peroxidase: ca. 3.5-10.0 mM

Molecular weight: ca. 43,000 (SDS-PAGE)

Isoelectric point: ca. ~~3-5~~ 4.5

Please replace the paragraph at column 10, lines 1-31, with:

Table 1 shows the purification performed so far. Table 2 shows physicochemical properties of the creatine amidinohydrolase obtained by the above methods.

TABLE 1  
Purification of creatine amidinohydrolase from *Escherichia coli*  
JM109 (pCRH273M1)

Step	Total activity (U)	Specific activity (U/mg-protein)	Yield (%)
French press rupture	52200		100.0
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> precipitation- redissolution	49746	8.3	95.3
Sephadex G-25	46927	10.3	89.9
Octyl Sepharose CL-6B	33094	18.4	63.4

TABLE 2  
Physicochemical properties of creatine amidinohydrolase purified  
from *Escherichia coli* JM109 (pCRH273M1)

Item	Physicochemical properties
Action	creatine + H <sub>2</sub> O → sarcosine + urea
Optimal temperature	ca. 40° C-50° C
Optimal pH	ca. 8.0-9.0
Thermal stability	ca. 50° C (50 mM potassium phosphate buffer, pH 7.5, 30 min treatment)
pH stability	ca. 5-8 (40° C, 18 hr preservation)
Km value	ca. 6.5 mM (creatine)
Molecular weight	ca. 43,000 (SDS-PAGE)
Isoelectric point	ca. 3.5-4.5 (isoelectric focusing)

Please replace the paragraph at column 12, line 65-column 11, line 29, with:

Table 3 shows the purification performed so far. Table 4 shows physicochemical properties of the creatine amidinohydrolase obtained by the above methods.

TABLE 3  
Purification of creatine amidinohydrolase from *Escherichia Coli*  
JM109 (pCRH273M2)

Step	Total Activity (U)	Specific activity (U/mg-protein)	Yield (%)
French press rupture	33600		100.0
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> precipitation- redissolution	25636	7.2	76.3
Sephadex G-25	24326	9.8	72.4
Octyl Sepharose CL-6B	19689	14.3	58.6

TABLE 4  
Physicochemical properties of creatine amidinohydrolase purified  
from *Escherichia coli* JM109 (pCRH273M2)

Item	Physicochemical properties
Action	creatine + H <sub>2</sub> O → sarcosine + urea
Optimal temperature	ca. 45° C-50° C
Optimal pH	ca. 8.0-9.0
Thermal stability	ca. 40° C (50 mM potassium phosphate buffer, pH 7.5, 30 min treatment)
pH stability	ca. 5-8 (40° C, 18 hr preservation)
Km value	ca. 4.5 mM (creatine)
Molecular weight	ca. 43,000 (SDS-PAGE)
Isoelectric point	ca. 3.5 <u>4.5</u> (isoelectric focusing)

Please replace the paragraph at column 11, line 65-column 12, line 29, with:

Table 5 shows the purification performed so far. Table 6 shows physicochemical properties of the creatine amidinohydrolase obtained by the above methods.

TABLE 5  
Purification of creatine amidinohydrolase form *Escherichia Coli*  
JM109 (pCRH273M3)

Step	Total activity (U)	Specific activity (U/mg-protein)	Yield (%)
French press rupture	49800		100.0
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> precipitation- redissolution	43027	8.3	86.4
Sephadex G-25	39989	9.9	80.3
Octyl Sepharose CL-6B	32021	14.8	64.3

TABLE 6  
Physicochemical properties of creatine amidinohydrolase purified  
from *Escherichia coli* JM109 (pCRH273M3)

Item	Physicochemical properties
Action	creatine + H <sub>2</sub> O → sarcosine + urea
Optimal temperature	ca. 40° C-45° C
Optimal pH	ca. 8.0-9.0
Thermal stability	ca. 40° C (50 mM potassium phosphate buffer, pH 7.5, 30 min treatment)
pH stability	ca. 5-8 (40° C, 18 hr preservation)
Km value	ca. 9.0 mM (creatine)
Molecular weight	ca. 43,000 (SDS-PAGE)
Isoelectric point	ca. <del>3.5</del> <u>4.5</u> (isoelectric focusing)